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## DISTRIBUTION OF CATHEPSIN D GRANULES IN NORMAL AND PATHOLOGIC CONDITIONS IN HUMAN PROSTATE

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Although cathepsin D has been implicated in prostate cancer invasion and metastasis, the distribution of this enzyme in normal human prostate is unknown. We investigated immunohistochemically the distribution of cathepsin D granules in normal prostatic tissues with or without androgen ablation. We also examined the cancer tissues after androgen ablation and the hyperplastic tissues. Changes in the distribution pattern of the larger cathepsin D-positive granules (apoptotic bodies) were observed in the normal prostate as well as in the normal tissue of the anti-androgen-treated cancer specimens. While the apoptotic bodies were denser in the proximal duct of the normal adult prostate, they were more abundant in the normal peripheral acini of the anti-androgen-treated cases. There were few apoptotic bodies in the adenoma tissues, but many in the hormonally treated cancer tissues. These results showed that the distribution pattern and density of cathepsin D granules well reflected the status of the human prostatic cells in relation to age, hormonal environment and hyperplastic or neoplastic change.

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**Key words:** Cathepsin D, Immunohistochemistry Apoptotic bodies, Prostate

### INTRODUCTION

Cathepsin D is an aspartyl lysosomal protease, which is found in a wide variety of mammalian cells<sup>1)</sup>. The physiological role of cathepsin D remains to be elucidated. Several investigators suggested its involvement in some important biochemical processes, such as secretion, activation and catabolism of other cellular proteins regulated by several factors<sup>2,3)</sup>.

In some conditions, the regulatory process is lost and there is an increase in extra-lysosomal release and/or increase in the activity of cathepsin D leading to the dissolution and degradation of extracellular matrix. This results in disruption of normal host cell barriers and cell death, and in a malignancy, results in invasion and metastasis of tumor cells. Thus overactivity of cathepsin D has been implicated in various cancer cells<sup>4,5)</sup>.

Maker et al. and Ross et al. suggested that overexpression of cathepsin D could be a useful predictor of disease progression or outcome of prostate cancer<sup>6,7)</sup>. However, despite the suggested role of cathepsin D in prostate cancer invasion and metastasis, little is known about the pattern of distribution of this enzyme in the normal prostate. We studied the distribution of cathepsin D granules in the normal prostate and compared it with the normal part of the cancer specimen from patients who received anti-androgen therapy before radical prostatectomy.

### MATERIALS AND METHODS

A series of 44 human prostates were used for immunohistochemical analysis of cathepsin D distribution. The prostates were taken from surgical and autopsy cases aged from 2 months to 86 years. The surgical specimens were from 20 cases of radical prostatectomy, 9 cases of cystoprostatectomy and 2 cases of subcapsular prostatectomy. Radical prostatectomy was performed after preoperative hormonal therapy (anti-androgen therapy or total androgen blockade therapy) for 3 to 6 months.

All the specimens were fixed in 10% buffered formalin and embedded in paraffin after cutting at 5 mm intervals perpendicular to the urethral axis from the apex to the base of the prostate. The 3  $\mu$ m-thick sections from the blocks were stained with hematoxylin and eosin. Immunohistochemistry was performed by the indirect immunoperoxidase method using rabbit polyclonal anti-cathepsin D (1 : 400; DAKO, Glostrup, Denmark). Briefly, sections were dewaxed and rehydrated, and endogenous peroxidase activity was blocked with hydrogen peroxide/methanol. Prior to immunostaining, the antigen was retrieved by microwaving. The tissue sections were blocked with 1 : 20 normal bovine serum albumin in tris-buffered saline (TBS; 140 mmol/l NaCl, 50 mmol/l Tris /HCl, pH 7.6) and incubated for 2 hours with the primary antibody. After washing in TBS, slides were then incubated with

horseradish peroxidase-labeled second antibody for 30 minutes. To visualize the immunostaining, we used diaminobenzidine tetrahydrochloride (DAB; Dojin Chemical, Tokyo, Japan) as a substrate, and counterstained tissue sections with hematoxylin. Then they were dehydrated and mounted. The primary antibody was omitted for the negative control. A known positive tissue control was used during staining.

The prostates were classified into the following groups: group 1 (n=5; prepubertal prostates of 2 months to 14 years old, mean age 6 years), group 2 (n=17; adult prostates of 20 to 86 years old, mean age 51.6 years), group 3 (n=20; adult prostates with hormonal therapy from 52 to 78 years old, mean age 68.3 years) and subcapsular adenomas (n=2).

Cathepsin D granules were observed from the anatomical viewpoint with special reference to the ductal-acinous structures. When a distinction between cancer tissue and distorted glands after the hormonal therapy was necessary, we applied keratin 34 beta E 12 staining for the differential diagnosis.

For a comparative study of larger cathepsin D granules (apoptotic bodies), we counted their number in the verumontanum, main prostatic duct and in peripheral acini of each prostate. In each region, at least 5 high-power-fields ( $\times 400$ ) were selected randomly for counting the apoptotic bodies. The values are presented as mean  $\pm$  SEM (standard error of the mean). A paired student's t-test was applied to the comparative study among different regions of the same group. A probability level of less than 0.05 was regarded as statistically significant.

## RESULTS

Two types of immunoreactions were principally observed in all the specimens examined: in one type, the cathepsin D positive granules were fine, and found to be situated in the apical or perinuclear cytoplasm of the secretory cells except in adenomatous tissues, while in the other type they were relatively larger (apoptotic bodies) and found on the luminal surface or shedding into the lumen of the ducts or acini.

Although fine cathepsin D granules were consistently but unevenly present in the luminal cells of all the specimen examined, there were regional variations in apoptotic bodies in different regions of the prostate with and without hormone therapy. Table 1 summarizes the distribution of the apoptotic bodies in various regions of the prostate. In the normal adult prostate (group 2), apoptotic bodies were noticed in the epithelial cells of verumontanum (Fig. 1a), utricle (Fig. 1b), prostatic urethra and ejaculatory ducts. Apoptotic bodies were significantly more abundant in the proximal part of the main prostatic ducts (Fig. 1c) than the distal part of ducts and the acini (Fig. 1d). There were changes in

Table 1. Distribution of apoptotic bodies in different regions of the prostate

Group	Verumontanum	Proximal ducts	Peripheral acini
Group 1	0.12 $\pm$ 0.10	0.08 $\pm$ 0.10	0.04 $\pm$ 0.08
	Ns	Ns	
Group 2	1.74 $\pm$ 0.32	1.18 $\pm$ 0.37	0.68 $\pm$ 0.30
	**	**	
Group 3	1.50 $\pm$ 0.22	1.92 $\pm$ 0.25	3.50 $\pm$ 0.51
	**	**	

Group 1 (n=5, prepubertal prostate), Group 2 (n=17, adult prostate), Group 3 (n=20, adult prostate with hormone therapy). Ns: Not significant, \*\*  $p \leq 0.01$

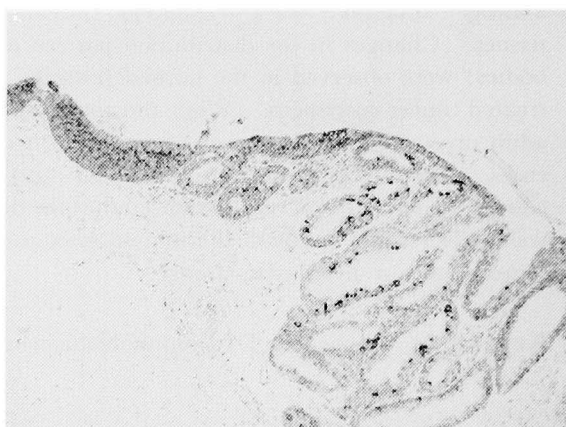


Fig. 1a. Apoptotic bodies were detected immunostaining for cathepsin D around the verumontanum in normal adult prostate.  $\times 100$ .

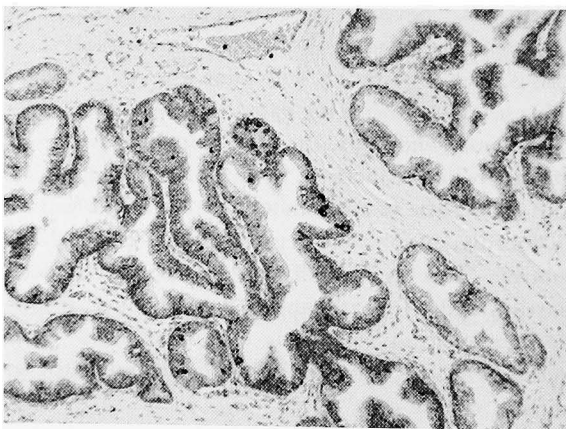


Fig. 1b. Many apoptotic bodies are observed in the utricle in normal adult prostate.  $\times 100$ .

the location of the apoptotic bodies in hormonally treated cases (group 3). The peripheral part of the ducts or the acini exhibited significantly more apoptotic bodies (Fig. 2a) than the proximal ducts and the utricle (Fig. 2b). In infants and in prepubertal prostate (group 1), fine cathepsin D granules were constantly observed, and apoptotic bodies were rarely seen in these specimens (Fig. 3).

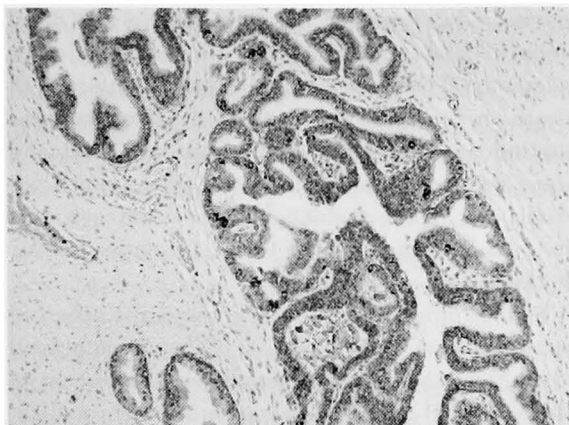


Fig. 1c. Many apoptotic bodies are observed in the proximal part of the duct in normal adult prostate.  $\times 100$ .



Fig. 2b. Very few apoptotic bodies are found around verumontanum of the hormonally treated prostate.  $\times 100$ .

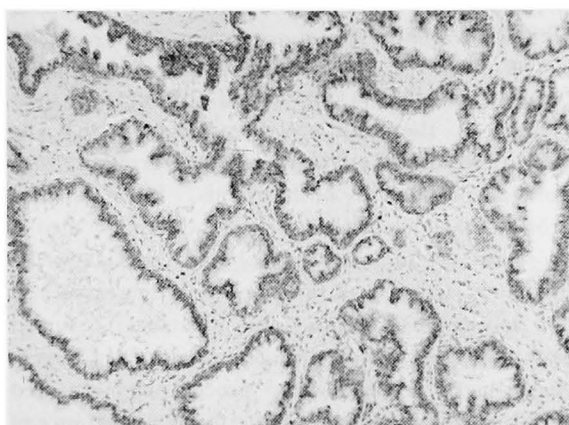


Fig. 1d. Very few apoptotic bodies are found in the distal part of duct and acini in normal adult prostate.  $\times 100$ .

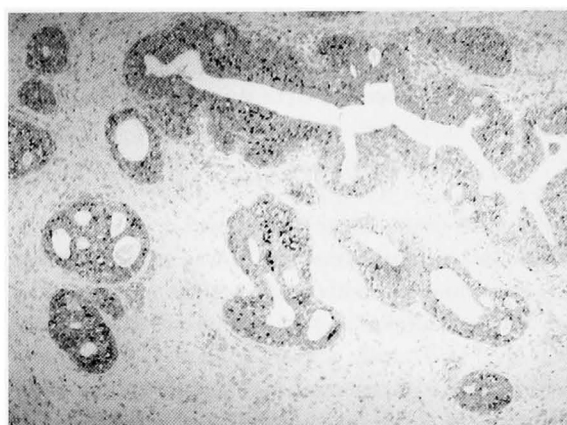


Fig. 3. Infant prostate: Fine cathepsin D granules are stained with antibody to cathepsin D. Apoptotic bodies are almost absent.  $\times 100$ .

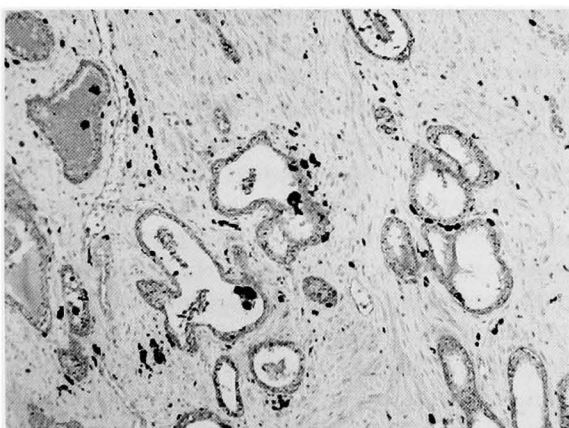


Fig. 2a. Enormous apoptotic bodies are visualized in the peripheral duct/acini of the hormonally treated prostate.  $\times 100$ .

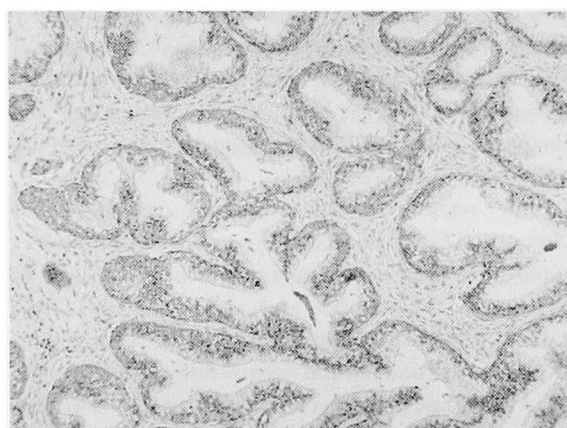


Fig. 4. Fine cathepsin D granules are present near the base of the luminal cells in the adenoma tissue, while apoptotic bodies are very few.  $\times 100$ .

In the adenomatous tissues of the subcapsular prostatectomy specimen, fine cathepsin D granules were contained mainly in the basal part of the cytoplasm of the luminal cells without notable apoptotic bodies in the epithelium or stromal tissues (Fig. 4), while enormous apoptotic bodies were

detected in the cancer tissues of the hormonally treated adenocarcinoma cases (Fig. 5). These apoptotic bodies were mainly located along the luminal surface or extruded into the lumen and also found in stromal tissues. The density of the

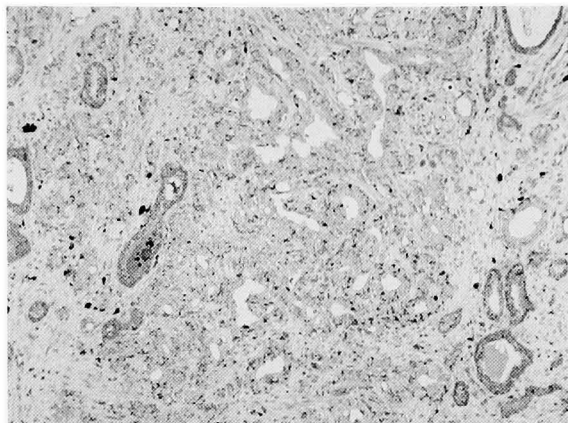


Fig. 5. Numerous apoptotic bodies are seen in the cancer tissues after hormonal treatment. They are present along the luminal surface or extruded into the lumen and in the stroma.  $\times 100$ .

apoptotic bodies seemed to be unrelated to the tumor grade.

### DISCUSSION

From the results of the study it was evident that the lysosomal enzyme cathepsin D was present at low concentrations unevenly in all regions of the normal prostate. Whitaker et al. also noticed the existence of cathepsin D with a qualitative and quantitative difference in its distribution among different organs or cell types of the same organ of normal rat<sup>8)</sup>. The reason for uneven pattern of cathepsin D distribution was not fully understood. However, Cherry et al. observed that cathepsin D caused hydrolysis of the PSA protein present in normal, BPH and cancer tissues<sup>9)</sup>. They showed that this catalytic activity was greater in cancer specimen than in normal and BPH specimen, and that the hydrolysis mediated by cathepsin D might have some impact on the fluctuation of serum PSA level found in cancer of the prostate.

With regard to the enhanced expression of cathepsin D, Sensibar et al. presumed that in the initial phase of programmed cell death there was an increase in the number and size of the positively stained granules in the affected cells, which were subsequently coalesce and occupied the major portion of the cytoplasm, and eventually led to the formation of larger granules<sup>10)</sup>. These larger granules gave rise to enhanced expression and represent the late manifestation of cell death (apoptotic cell death).

We also noticed the presence of apoptotic bodies in normal tissues and in tissues from patients who received anti-androgen therapy. Our results showed that there were regional variations in functional activities of the epithelial cells lining the prostatic ductal and acinar systems. While the proximal ductal epithelial cells were more apoptotic in the

normal adult prostate, the epithelium of the distal segment and acini followed this after androgen ablation. This suggested that programmed cell death also occurred frequently in the ducts near the urethra in normal circulating androgen level and there was a reversal in the site of programmed cell death after androgen ablation. Thus androgen must have some regulatory role in prostatic cellular homeostasis.

Similar programmed cell death was also observed in rat prostate by Lee et al.<sup>11)</sup> They hypothesized that the mechanism for maintaining homeostasis of the epithelial cell population in the normal rat prostate was achieved by having cell proliferation in distal ductal segments and cell degeneration in proximal ductal segments, and there was a reversed shift of location of both proliferation and degeneration after castration.

The absence of larger cathepsin D granules (apoptotic bodies) in prepubertal prostates and their presence in the adult prostates suggested that the appearance of these bodies was well correlated with the biological activity of the glands. In prepubertal prostates the biological activity of the gland seemed to be static, while in postpubertal prostates the functional activities of the gland were increased and the development of the acinar structures occurred probably due to the elevated level of androgen. Accordingly, a degenerated process also occurred mainly in the proximal ducts with the appearance of apoptotic bodies to maintain morphological and functional homeostasis of the whole prostate. Conversely, the reversed directional changes were observed due to oversuppression of androgen activity.

Similar to the prepubertal prostate, the apoptotic bodies were absent in the adenomatous tissues, which might suggest that the glands in adenomas maintained a relatively static activity. It was speculated that benign prostatic hyperplasia (BPH) was associated with an increase in prostatic volume which results from either an increase in cell proliferation with unchanged cell death or with unchanged cell proliferation and less cell death<sup>12)</sup>. Claus et al. showed that the induction of BPH from normal prostate was obviously associated with a distinct increase in cell proliferation in epithelium and in stroma<sup>13)</sup>. The absence or decrease in number of apoptotic bodies in the adenoma tissue in our study supported the hypothesis that in the development of adenoma, programmed cell death process was reduced or did not take part. Djonov et al. also recently noticed a close relationship between down regulation of transforming growth factor (TGF)- $\beta$  and the decrease in number of apoptotic bodies in BPH<sup>14)</sup>. However, the reason for the basal location of the fine cathepsin D granules in the luminal cells was not clear and further study is required to draw a

conclusion with a large series of BPH specimens.

In relation to the immunohistochemical characteristics of cathepsin D in prostate cancer, Maygarden et al. noted that 96 of 102 prostate cancers expressed at least some degree of cathepsin D positivity and there was a significant association with Gleason score<sup>15)</sup>. On the other hand, Marker et al. found 39 of 78 prostate carcinomas expressing cathepsin D positively and they had noticed a non-significant association with Gleason score, but a significant relationship with pathological stage<sup>6)</sup>. In our study, all the cancer specimens showed a strong reactivity to cathepsin D with enormous apoptotic bodies. The difference might be attributed to the effect of the preoperative hormonal therapy in our series. At least, certain populations of prostate cancer cells were androgen dependent and they had undergone apoptosis in response to androgen withdrawal leading to involution of the cancer cells.

In conclusion, the density of apoptotic bodies was well correlated with the changes of the condition of the human prostatic tissues.

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## 和文抄録

## ヒト前立腺におけるカテプシンD顆粒の分布

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蛋白分解酵素であるカテプシンDは前立腺癌の浸潤，転移に関与するといわれているが，この酵素の正常前立腺での分布に関しては知られていない。われわれは免疫組織化学的にこの酵素の分布を正常前立腺およびホルモン療法後の前立腺全摘後の標本で癌組織のない部分でその相違を検討した。さらに肥大症組織およびホルモン療法後の癌組織部でもその発現を検討した。

カテプシンD顆粒は細かい顆粒といわれるアポトーシス体とよばれる大きな顆粒の2種類に分類でき，前者は前立腺上皮に不均一であるが普遍的に認められた。後者のアポトーシス体は成人前立腺では尿道近位側の主導管部，男性子宮および精阜上皮に比較的多く

認めるが，末梢腺房上皮では認めなかった。一方，ホルモン療法後の非癌組織ではこの傾向は逆転し，末梢腺房部で多く認め，主導管や男性子宮での発現は減少していた。またアポトーシス体は思春期前の前立腺や前立腺肥大症組織では認めなかった。ホルモン療法後の癌組織ではかなりのアポトーシス体をその腺腔内や間質に認めた。

これらの結果より，カテプシンDの分布，とりわけアポトーシス体の分布はヒト前立腺上皮細胞の活動性および退行性をよく反映し，さらに内分泌環境の変化をよく反映した。またアポトーシス体は加齢や肥大症，癌化に伴い，その発現性が変化した。

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